JACC Vol. 30, No. 2
August 1997:576–84

Repolarization Abnormalities, Arrhythmia and Sudden Death in Canine Tachycardia-Induced Cardiomyopathy

PETER H. PAK, MD, H. BRADLEY NUSS, PHD, RICHARD S. TUNIN, MS, STEFAN KÄÄB, PHD, GORDON F. TOMASELLI, MD, FACC, EDUARDO MARBAN, MD, PHD, FACC, DAVID A. KASS, MD

Baltimore, Maryland

Objectives. **This study sought to determine whether the canine model of tachycardia-induced heart failure (HF) is an effective model for sudden cardiac death (SCD) in HF.**

Background. **Such a well established HF model that also exhibits arrhythmias and SCD, along with repolarization abnormalities that could trigger them, may facilitate the study of SCD in HF, which still eludes effective treatment.**

Methods. **Twenty-five dogs were VVI-paced at 250 beats/min for 3 to 5 weeks. Electrocardiograms were obtained, and left ventricular endocardial monophasic action potentials (MAPs) were recorded at six sites at baseline and after HF. Weekly Holter recordings were made with pacing suspended for 24 h.**

Results. **Six animals (24%) died suddenly, one with Holter-documented polymorphic ventricular tachycardia (VT). Holter recordings revealed an increased incidence of VT as HF progressed. Repolarization was significantly ($p < 0.05$) prolonged, as indexed by a corrected QT interval (mean [±SD] 311 ± 25 to 338 ± 25 ms) and MAP duration measured at 90% repolarization ($MAPD_{90}$) (181 ± 19 to 209 ± 28 ms), and spatial $MAPD_{90}$ dispersion rose by 40%. We further tested whether CsCl inhibition of repolarizing K^+ currents, which are reportedly downregulated in HF, might preferentially prolong the $MAPD_{90}$ in HF. With 1 mEq/kg body weight of CsCl, $MAPD_{90}$ rose by 86 ± 100 ms in dogs with HF versus only 28 ± 16 ms in control animals ($p = 0.002$). Similar disparities in CsCl sensitivity were observed in myocytes isolated from normal and failing hearts.**

Conclusions. **Tachycardia-induced HF exhibits malignant arrhythmia and SCD, along with prolonged, heterogeneous repolarization and heightened sensitivity to CsCl at chamber and cellular levels. Thus, it appears to be a useful model for studying mechanisms and therapy of SCD in HF.**

(J Am Coll Cardiol 1997;30:576–84)

Sudden cardiac death (SCD) accounts for 23% to 52% of total mortality in patients with dilated cardiomyopathy (1–3). Although ventricular arrhythmias are responsible for a majority of these deaths, the underlying mechanisms remain poorly understood (4,5). Repolarization abnormalities demonstrated in isolated myocytes (6–9) and enhanced dispersion of repolarization may render the heart vulnerable to nonexcitable gap reentry (10), increasing the probability of afterdepolarizations, which can induce triggered arrhythmias (11). Reduced outward K^+ currents, notably transient outward current (I_{to}) and inward rectifier current (I_{K1}) (9) may contribute to action potential prolongation.

Although human studies are valuable, failing cardiac tissue is typically obtained only at end-stage with marked heterogeneity of disease duration, etiology and previous treatment. Thus, a controlled animal model with fatal arrhythmia and cellular repolarization abnormalities analogous to those observed in human disease would be useful for exploring mechanisms and treatments of heart failure (HF) related sudden death. A promising animal model is that induced by pacing tachycardia in the dog (12–17), which displays mechanical (12–14), biochemical (12,15,16) and molecular abnormalities similar to HF in humans (17). We recently reported that this model also displays markedly reduced I_{to} and action potential prolongation in failing myocytes (18).

The present study tested whether tachycardia pacing–induced HF also exhibits prolonged and spatially heterogeneous repolarization at the chamber level, whether it is also associated with malignant arrhythmia and sudden death and whether there is an increased sensitivity of repolarization to cesium chloride (CsCl), a compound known to inhibit repolarizing currents. This would be important because it would support the notion that the demonstrated cellular repolarization defects are associated with profoundly altered electrophysiologic substrate at the chamber level.

From the Division of Cardiology, Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, Maryland. This study was supported by grants HL P50-52307 (Specialized Center of Research in Sudden Cardiac Death) and RO1-HL47511 (Dr. Kass) from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland, and by an American College of Cardiology/Merck Fellowship (Dr. Pak) and an American Heart Association Established Investigator Award (Dr. Kass).

Manuscript received September 7, 1996; revised manuscript received April 14, 1997, accepted April 25, 1997.

Address for correspondence: Dr. Peter H. Pak, Halsted 500, Division of Cardiology, Johns Hopkins Medical Institutions, Baltimore, Maryland 21287. E-mail: peterpak@welchlink.welch.jhu.edu.

0735-1097/97/$17.00
PII S0735-1097(97)00193-9

Abbreviations and Acronyms

APD_{90}	=	action potential duration at 90% repolarization
dP/dt	=	rate of rise of left ventricular pressure
HF	=	heart failure
I_{Kl}	=	inward rectifier current
I_{to}	=	transient outward current
LVEDP	=	left ventricular end-diastolic pressure
MAP (D_{90})	=	monophasic action potential (duration at 90% repolarization)
NSVT	=	nonsustained ventricular tachycardia
SCD	=	sudden cardiac death
VT	=	ventricular tachycardia

Methods

Study group. Twenty-five adult mongrel dogs of either gender underwent sterile surgical instrumentation for rapid ventricular pacing. Seventeen dogs were instrumented for conscious hemodynamic recording (thoracotomy pacing group), and eight dogs were instrumented solely with a transvenous pacemaker system and subcutaneous electrocardiographic (ECG) leads (transvenous pacing group). Monophasic action potentials (MAPs) were also measured in 10 nonpaced control dogs before myocyte isolation for cellular electrophysiologic studies. Not all tests were performed in each animal. Animal protocols were approved by the Johns Hopkins Animal Care and Use Committees and conformed to the "Position of the American Heart Association on Research Animal Use," adopted by the Association in November 1984.

Instrumentation. Animals in the transvenous pacing group were sedated with butorphanol tartrate (4 mg) and xylazine (2.2 mg/kg body weight) intramuscularly, and a bipolar endocardial lead (Medtronic, Inc.) was placed under fluoroscopic guidance at the right ventricular apex through the left internal jugular vein using local anesthesia. A programmable pacemaker (Activitrax, Spectrax or Legend by Medtronic, Inc.), with a magnet glued to the canister to disable the upper-rate limit without altering ventricular sensing, was linked to the lead and inserted into a subcutaneous pocket at the base of the neck. Subcutaneous ECG leads were sewn in place and exteriorized for subsequent Holter monitoring. Dogs were provided at least 2 days for recovery before initiating pacing.

Animals in the thoracotomy pacing group underwent a left lateral thoracotomy under barbiturate and halothane anesthesia, and epicardial pacing leads were attached to the right ventricular free wall. The modified pacemaker was placed in a subcutaneous pocket and attached to the pacing lead. A left ventricular tube for introducing a micromanometer was inserted through an apical stab and secured by purse-string suture. Sonomicrometer crystals were placed at the mid left ventricular level to provide anteroposterior dimension. All wires, catheters and tubes were exteriorized at the midscapular site. Sonomicrometer wires also served as intracardiac leads for continuous ambulatory ECG monitoring. At least 11 days were provided for full surgical recovery.

Experimental protocol. Baseline hemodynamic (n = 12) and surface ECG (n = 16) data were collected in the conscious state in the 17 dogs in the thoracotomy pacing group. For MAP recordings, all animals were first sedated with butorphanol and xylazine, and arterial access was obtained through sterile femoral artery cutdown under local anesthesia. A steerable catheter (EP Technologies, model 644001) was used to measure MAPs at six different sites in the left ventricular cavity: apical, mid septal, high septal, anterior, high lateral and low lateral walls. Signals were low pass filtered at 0.3 Hz, and high pass filtered at 300 Hz. In addition to steady-state MAP duration (n = 21), the sensitivity of MAP duration measured at 90% repolarization ($MAPD_{90}$) to CsCl-induced inhibition of repolarizing K^+ currents (19–21) was determined in a subset of 11 animals. Monophasic action potentials at a single, stable site were recorded before and after incremental doses of intravenous CsCl (0.25 mEq/kg per dose) delivered over 15 s at 5-min intervals. Heart rate was kept constant during CsCl infusion by pacing the left ventricle through the MAP catheter at a rate 10% higher than the baseline heart rate. This dosing scheme was chosen to unmask higher sensitivity of $MAPD_{90}$ prolongation in dogs with HF while not provoking significant hemodynamic alterations or torsade de pointes, as higher doses of CsCl would (22). After data recordings were complete, the femoral arteriotomy was repaired, the incision was closed and the animal was allowed to fully recover.

Pacing was initiated at 250 beats/min in the VVI mode on the day after baseline recording. In seven animals (six in the thoracotomy pacing group and one in the transvenous pacing group), pacing was suspended (VVI pacing at 30 beats/min at the lowest possible energy output from the pulse generator) for 24 h at weekly intervals, and Holter monitor recordings were made during this period. After at least 3 weeks of pacing (mean 30 ± 9 days), often when the animal exhibited outward clinical signs of HF, hemodynamic and electrophysiologic data were again obtained (HF time point). Conscious left ventricular pressure–dimension data (n = 9) and ECGs (n = 13) were obtained in the animals in the thoracotomy pacing group. Left ventricular pressure measurements were also made in six of eight animals in the transvenous pacing group at the time of the terminal study to confirm the presence of HF. Monophasic action potential recordings were made in a total of 11 animals after development of HF, and CsCl infusion tests were completed in four of these dogs. To further test the relation between ECG and hemodynamic changes induced by rapid pacing, data were also examined after 1 week of pacing in 12 dogs in the thoracotomy pacing group.

Myocyte isolation. The harvesting of viable myocytes required rapid excision of the heart after controlled cardioplegia and was not easily achieved in the dogs in the thoracotomy pacing group because of scarring. Therefore, HF myocytes were derived from the animals in the transvenous pacing group, whereas control myocytes were obtained from dogs that were never paced. Dogs were anesthetized with pentobarbital

(30 mg/kg intravenously) and intubated, and the heart was exposed by left thoracotomy. Heparin (10,000 U intravenously) was injected, and iced lactated Ringer's solution was placed within the chest cavity. After ligating the aortic arch vessels, the venae cavae and the proximal descending aorta, cold cardioplegic solution (modified St. Thomas solution) was retroperfused to induce cardiac arrest. The heart was rapidly excised and immersed in cold cardioplegic solution.

Ventricular myocytes were isolated as previously described (18,23). The left anterior descending coronary artery was cannulated and perfused at 15 ml/min by 30 min in a nominally Ca^{++}-free modified Tyrode's solution containing (in mol/liter) 138 of NaCl, 4 of KCl, 1 of $MgCl_2$, 10 of glucose, 0.33 of NaH_2PO_4 and 10 of HEPES, at pH 7.3, followed by 40 min in the same solution with collagenase (type I, 178 U/ml, Worthington Biochemical Corp.) and protease (type XIV, 0.12 mg/ml, Sigma Chemical Co.). The enzyme solution was recirculated. The myocardial segment was then washed for 15 min with modified Tyrode's solution containing 200 μmol/liter of Ca^{++}. All solutions were oxygenated with 100% oxygen and warmed to 37°C. To control for the previously described transmyocardial variability of the currents (24,25), all cells were isolated from the central third of the myocardial wall. At the end of perfusion, endocardial and epicardial layers were dissected from the myocardial segment, and small chunks of well digested midmyocardial tissue were mechanically disaggregated, filtered through a nylon mesh and stored at room temperature in Tyrode's solution containing 2.0 mmol/liter of Ca^{++} until electrophysiologic study. Only Ca^{++}-tolerant cells with clear cross striations and without spontaneous contraction or significant granulation (~20% to 40% of those isolated in both control and failing hearts) were selected for experiments.

Electrophysiologic recording techniques. The whole-cell configuration of the patch-clamp technique was employed (26). Myocytes were transferred to the stage of an inverted microscope and superfused at a rate of 1 to 2 ml/min with the external solution containing (in mmol/liter) 136 of NaCl, 4 of KCl, 2.0 of $CaCl_2$, 1 of $MgCl_2$, 10 of glucose, 2 of sodium pyruvate and 10 of HEPES, at pH 7.4. Pipettes were filled with a solution containing (in mmol/liter) 140 of KCl, 1 of $MgCl_2$, 4 of MgATP, 10 of HEPES and 5 of NaCl, at pH 7.4. Action potential recordings were performed at 37°C using the voltage follower mode of an Axoclamp 2A voltage-clamp amplifier (Axon Instruments) at a cycle length of 2,000 ms and sampled at 1 to 2 kHz through a Digidata 1200 A/D (Axon Instruments) interface for off-line analysis. The susceptibility of action potential duration to the CsCl-induced inhibition of repolarizing K^+ currents was assessed by adding 1 mmol/liter of CsCl to the recording solution.

Data analysis. Sudden cardiac death was defined as death occurring in dogs that did not show any outward clinical signs of HF (lethargy, anorexia, tachypnea, ascites and edema) or other disease processes within 24 h of their determined demise, and in whom a gross autopsy revealed no other obvious cause of death. Even without outward clinical signs of HF, the animals may have had depressed systolic function and elevated filling pressures. Holter monitor recordings were scanned using a Del Mar analyzer (model 750), and the incidence of nonsustained ventricular tachycardias (NSVT) was determined each week during the evolution of HF. Ventricular tachycardia (VT) was defined as three or more consecutive beats of ventricular origin at a rate of 100 beats/min or higher.

Data analysis of hemodynamic and ECG signals was performed using signal-averaged waveforms derived from 5 to 10 sequential steady-state cycles. The end-diastolic pressure was at the point of rapid pressure upstroke, and the maximal rate of rise of left ventricular pressure (dP/dt_{max}) was digitally calculated from a five-point weighted slope. Monophasic action potential recordings with stable rest voltage were selected for analysis. The duration of MAP at 90% repolarization was determined by an automated computer algorithm. At each recording site, five to six consecutive beats of MAP were signal averaged and the $MAPD_{90}$ determined. Spatial dispersion of $MAPD_{90}$ was determined by the equation:

$$\text{MAPD}_{90}\text{ dispersion} = \sum_{i=1}^{n} \frac{|\text{MAPD}_i - \overline{\text{MAPD}_{90}}|}{n \times \overline{\text{MAPD}_{90}}} \times 100,$$

where $MAPD_i$ is the $MAPD_{90}$ at an individual site; $\overline{MAPD_{90}}$ is the mean $MAPD_{90}$ for the whole ventricle; and n is the total number of MAP sites. This method obviated limitations of $MAPD_{90}$ dispersion calculations based on the difference between two extreme values (27,28) and is similar to the coefficient of variation method of Priori et al. (28).

Statistical analysis. Pooled data are presented as mean value ± SD unless specified otherwise. Heart failure data were compared with the baseline data using the two-tailed t test for two samples, with $p < 0.05$ considered significant. The effect of incremental CsCl injection on $MAPD_{90}$ at baseline and during HF was tested by repeated measures analysis of variance with the Fisher least significant difference test for pairwise multiple comparisons.

Results

Hemodynamic data and sudden death. Tachycardia pacing resulted in marked decreases in dP/dt_{max} (3,034 ± 799 vs. 1,965 ± 656 mm Hg/s, $p < 0.001$) and fractional shortening (21.6 ± 6.9% to 13.5 ± 5.4%, $p < 0.001$), as well as a 138% rise in left ventricular end-diastolic pressure (LVEDP) (from 13 to 28 mm Hg, $p < 0.001$). Hemodynamic evidence of HF was similar between the 12 dogs in the thoracotomy pacing group previously reported (17) and the six dogs in the transvenous pacing group (LVEDP 36 ± 3 mm Hg, dP/dt_{max} 1,187 ± 239 mm Hg/s).

Of the 25 dogs that entered the pacing protocol, 10 died before the protocol was completed. Six (24%) died suddenly, having no outward manifestations of pump failure. The lack of outward symptoms of HF served to differentiate these animals from the two that died of pump failure. Two additional deaths were related to complications of the preparation (noncardiac death). Four of the sudden deaths occurred during active

Figure 1. A, Example of VT recorded by Holter monitoring. The dog had been paced for 20 days, and this recording was made during a 24-h period without pacing to quantitate native ventricular arrhythmias. **B,** The terminal rhythm recorded from one dog that died suddenly while being monitored.

pacing—one occurred 10 days after pacing was suspended after induction of HF and one occurred after 2 weeks of pacing, during the 24 h that pacing was suspended for weekly Holter monitoring. All but one of the sudden deaths occurred after 2 weeks of pacing, so that cardiodepression and other hemodynamic changes were most likely present (12,14,16,17). The rate of sudden death in the thoracotomy pacing group (24%) and the transvenous pacing group (25%) was similar.

Electrocardiographic monitoring. Nonsustained ventricular tachycardia was more frequently observed in dogs with HF (Fig. 1A). The number of NSVT episodes per 24 h increased significantly from 0.4 ± 0.6 (n = 5, range 0 to 1) to 6.7 ± 10.2 (n = 6, range 0 to 27) with HF (p = 0.04). The mean duration of NSVT runs at HF was 18 ± 36 beats (mean rate 158 ± 28 beats/min). One dog died suddenly while being monitored, and the recording revealed the terminal event as polymorphic VT or ventricular fibrillation (Fig. 1B). Hemodynamic evidence of HF in the seven monitored dogs was quantitatively and statistically similar to that observed in the overall group (change in dP/dt_{max} −45% and change in LVEDP 154%).

Repolarization abnormalities. Table 1 provides electrophysiologic data at baseline and after HF. The heart rate–corrected QT interval (QTc) rose significantly by 8.7% with the development of HF (Table 1). This change was not evident after only 1 week of rapid pacing in 12 dogs (QTc 317.0 ± 32.6 ms, p = 0.38 vs. baseline) despite systolic impairment (percent change in dP/dt_{max} −28.6 ± 26.4% and percent change in fractional shortening −21.3 ± 16.9%, p < 0.001). However, the LVEDP rise was also minimal at this earlier time (change in LVEDP 4.1 ± 8 mm Hg, p = NS).

Prolongation of repolarization was also demonstrated by direct ventricular MAP recordings, which revealed lengthening of $MAPD_{90}$ by 15.5%. There was also a marked increase in the spatial dispersion of $MAPD_{90}$, from 3.8 ± 3.4% at baseline to 5.3 ± 3.7% during HF, an increase of 39.5% (p = 0.01) (Fig. 2). There were no significant differences in these variables between the subgroup that received Holter monitoring and the animals that were not monitored (QTc 8.4% vs. 8.7%, p = 0.58; $MAPD_{90}$ 16.2% vs. 15.5%, p = 0.90; MAP dispersion 59.7% vs. 39.5%, p = 0.58, respectively).

Response to CsCl. Prolonged repolarization could be due to a reduction in outward rectifying K^+ currents (9,18). This mechanism would predict an enhanced sensitivity of $MAPD_{90}$ to further inhibition of these currents by CsCl, a nonspecific blocker of repolarizing K^+ currents that can induce early

Table 1. Repolarization Changes With Heart Failure

	Baseline	HF	p Value
ECG data	n = 16	n = 13	
Heart rate (beats/min)	139.7 ± 23.9	140.4 ± 32.3	0.947
QRS complex (ms)	64.2 ± 13.2	67.9 ± 20.2	0.238
QT interval (ms)	200.9 ± 27.8	228.0 ± 28.1	0.055
QTc interval (ms)	310.9 ± 25.3	337.8 ± 25.1	0.002
MAP data	n = 21	n = 11	
Heart rate (beats/min)	132.6 ± 21.9	135.6 ± 9.1	0.680
$MAPD_{90}$ (ms)	181.1 ± 19.2	209.2 ± 28.1	0.002

ECG = electrocardiographic; MAP = monophasic action potential; $MAPD_{90}$ = monophasic action potential duration at 90% repolarization: QTc = corrected QT interval (QT/RR).

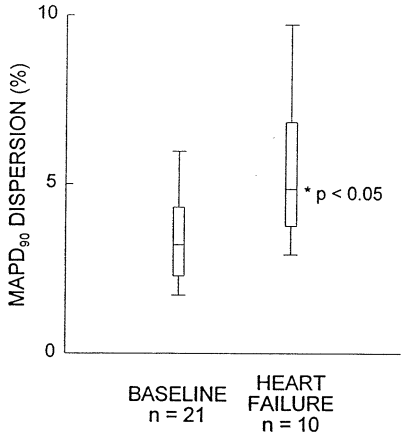

Figure 2. Box plots of $MAPD_{90}$ dispersion at baseline and during HF. The maximal and minimal points in each **box** show the 75th and 25th percentiles, respectively, and the **horizontal line** within the box shows mean $MAPD_{90}$. The **error bars** are at the 95th and 5th percentiles.

afterdepolarizations and, at sufficiently high doses, torsade de pointes (19–21,29,30). Figure 3 displays the results of these experiments, results which were consistent with this hypothesis. Cesium chloride did not significantly alter the $MAPD_{90}$ in the control dogs at baseline, but it markedly increased $MAPD_{90}$ by 43.5% in the dogs with HF ($p < 0.05$ by repeated measures analysis of variance).

Cellular electrophysiology. The high incidence of sudden death in HF and increased $MAPD_{90}$ in failing hearts may have been a reflection of an increased propensity to abnormal repolarization of individual myocytes. There was no difference in rest membrane potentials with HF (-81.9 ± 3.4 mV at baseline vs. -84.4 ± 4.4 mV during HF, $p = 0.20$, $n = 9$ in each group). However, similar to findings in the intact hearts, mean action potential duration at 90% repolarization (APD_{90}) was higher in the failing myocytes (468 ± 140 ms vs. 411 ± 75 ms in control hearts, $n = 9$ in each group), although this fell short of statistical significance due to intercell variability. Nevertheless, failing myocytes did demonstrate a significantly higher sensitivity of APD_{90} to CsCl (Fig. 4). The APD_{90} rose significantly ($p < 0.05$) in both control (148.2 ± 120.4 ms) and HF (281.7 ± 294.4 ms) myocytes, but this prolongation was nearly two times greater in failing cells ($p = 0.03$ by the Mann-Whitney *U* test). Figure 4A displays an example of the

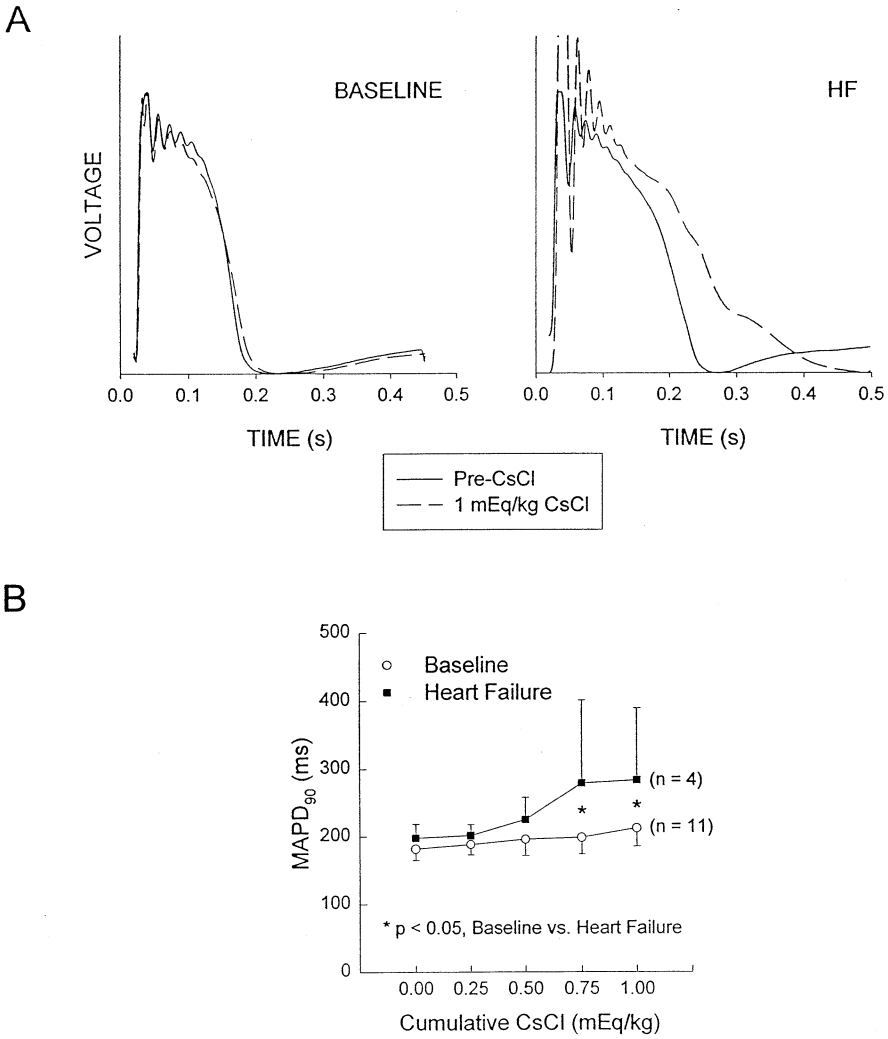

Figure 3. A, Monophasic action potential tracings from an animal at baseline and after induction of HF before **(solid lines)** and after **(dashed lines)** administration of a cumulative dose of 1 mEq/kg of CsCl. Cesium chloride had a minimal influence on MAP duration at baseline. After development of HF in the same animal, this CsCl dose markedly prolonged the MAP. **B,** Group data: $MAPD_{90}$–CsCl dose-response curves at baseline **(open circles)** and after development of HF **(solid squares)**. As in the example shown in **(A)**, there was no significant change in $MAPD_{90}$ with increasing CsCl doses at baseline, but there was substantial prolongation at higher CsCl doses during HF.

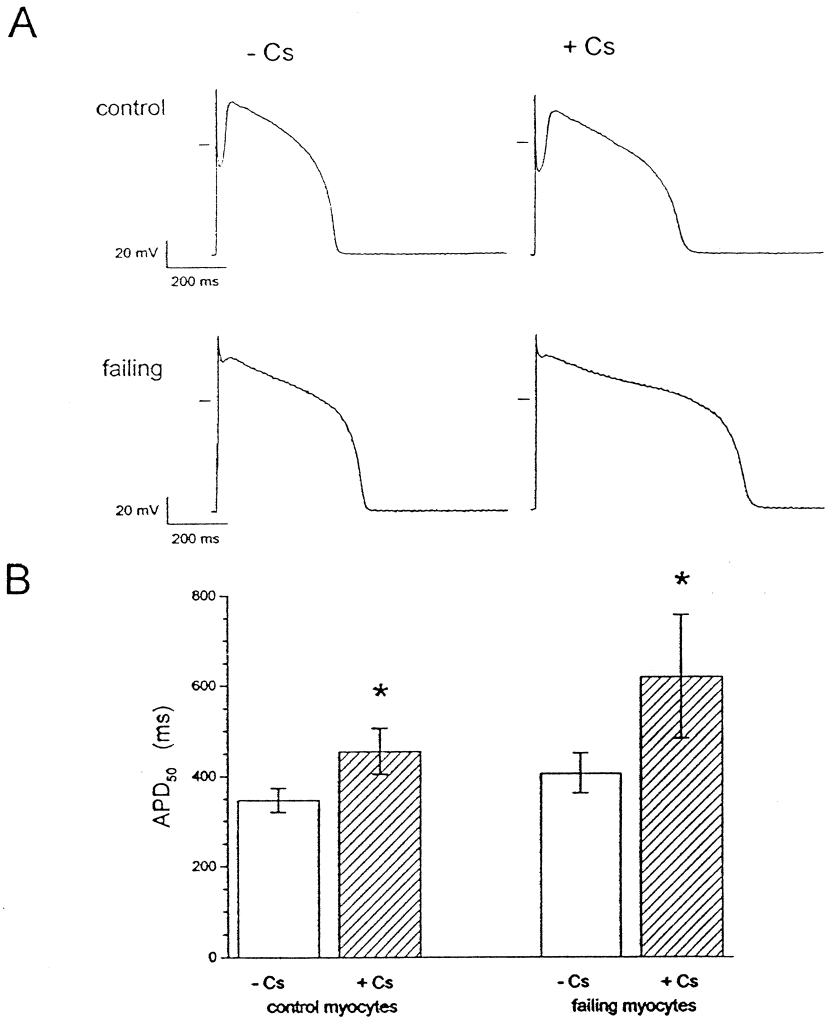

Figure 4. A, Action potential tracings from an isolated myocyte of a normal (control) heart and a failing heart. Data are shown both without and with 1 mmol/liter of CsCl (−Cs and +Cs, respectively). Cesium chloride increased the APD_{90} in both types of cells, but the response was far more pronounced in failing myocytes. **B,** Group data showing the effect of CsCl on APD_{90} at baseline and during HF. The **error bars** indicate SEM. As in the example, APD_{90} prolongation by CsCl was greater in myocytes isolated from failing hearts. *$p < 0.05$ versus −CsCl.

response to CsCl in myocytes from a control heart and a failing heart, and Figure 4B provides the group data for APD_{90}.

Discussion

Prolongation of repolarization has been reported previously in the tachycardia-induced model of HF (31–33). The present study provides novel evidence of additional abnormalities: enhanced spatial dispersion of repolarization, an increased proclivity to NSVT and sudden death and enhanced sensitivity of repolarization duration to CsCl. Although a mechanistic link between these phenomena could not be established by the present study, we suspect such a link is likely.

Significance of repolarization abnormalities. The corrected QT interval increased by 8.3% with development of HF, and this prolonged repolarization was also observed by lengthening of $MAPD_{90}$ in the absence of an increase in QRS duration. These data are consistent with previous results from the same animal model reported by Wang et al. (31), who found an 18.7% increase in $MAPD_{90}$ in isolated failing hearts, and Li et al. (32), who reported an 18.7% increase in APD_{90} by microelectrode studies on isolated papillary muscles. Consistent QTc lengthening has not been reported in humans with HF, but this may relate to higher variance due to concomitant drug therapy and a lack of direct pre-HF and post-HF ECGs (5). However, APD prolongation has been demonstrated in isolated myocytes from failing human myocardium (9), similar to what we found in the pacing canine model (18).

Prolongation of the action potential can yield a milieu favorable to ventricular arrhythmias by increasing heterogeneity of repolarization, leading to nonexcitable gap reentry (10), and by causing triggered arrhythmias through development of afterdepolarizations (11). In the long QT syndrome, the risk of sudden death is associated not only with lengthening of the QT interval, but also with increased heterogeneity of repolarization measured by QT dispersion (28,34). Increased dispersion of repolarization has been associated with electrical instability or sudden death, or both, with type I antiarrhythmic therapy (35), ischemic heart disease (36,37) and hypertrophic heart disease (38,39). Barr et al. (40) recently suggested that dispersion of repolarization may be associated with electrical instability and sudden death in HF. The present findings are consistent with these studies, and the magnitude of increased $MAPD_{90}$ dispersion is comparable to that reported in human

HF (5). Although we did not find the rest membrane potentials in the failing myocytes to be less negative than those in the control myocytes, Li et al. (32) reported that the failing myocytes were more depolarized at rest. This discrepancy may be due to our sample size and intercell variability. Less negative rest membrane potentials in failing myocytes would further increase the arrhythmogenic potential in the failing heart.

Mechanism of repolarization abnormalities. Abnormalities in serum electrolytes can contribute to repolarization abnormalities and ventricular arrhythmia, and reduced levels of K^+ and Mg^{++} are not uncommon in HF. However, animals were not treated with diuretic agents, and previous studies with this model have not revealed such electrolyte changes (32). We determined serum electrolytes in only a small subset of animals and found no changes with HF. Further confirmation was made in six subsequently studied dogs transvenously paced in a fashion identical to that in the present study. Again, we found no change in serum K^+ (3.6 ± 0.47 mmol/liter in control dogs and 3.7 ± 0.51 in those with HF, $p = 0.82$) and Mg^{++} (1.35 ± 0.23 mEq/liter in control dogs and 1.33 ± 0.19 in those with HF, $p = 0.84$), and a very small but statistically significant decline in ionized serum Ca^{++} (1.40 ± 0.06 mmol/liter in control dogs and 1.36 ± 0.07 in those with HF, $p = 0.03$). This, likely, is not responsible for repolarization abnormalities, because isolated myocyte APD_{90} measured at the identical $[Ca^{++}]_o$ also showed increased APD_{90} with heart failure. Hypothyroidism can also lead to action potential prolongation, but tachycardia-induced HF is associated with sick euthyroid syndrome and not frank hypothyroidism (41).

Other mechanisms for action potential prolongation with HF relate to abnormalities in specific ion channel currents. Beuckelmann et al. (9), studying explanted human heart tissue, reported a decrease in K^+ currents, notably I_{to}, in end-stage HF. We recently reported similar abnormalities in myocytes isolated from pacing-induced HF in dogs and showed this defect to be primarily due to a reduced number of functioning channels (18). Although the time when I_{to} is active is brief and occurs early in the action potential, decreasing I_{to} was capable of significantly prolonging the APD_{90} (18). The present study supports a major implication of such a current reduction that repolarization in failing hearts and isolated "failing" myocytes is far more sensitive to blockade of repolarizing K^+ currents, as induced by CsCl. This is important, because it indicates that previously documented cellular defects lead to a profoundly altered electrophysiologic substrate at the chamber level as well.

Canine tachycardia-induced dilated cardiomyopathy model. The canine pacing tachycardia-induced dilated cardiomyopathy model of HF has proven useful in that it reproducibly induces characteristic hemodynamic and biochemical (12–17,42) abnormalities. Molecular analysis of myocytes is possible (16,17,43), and therapeutic interventions can be tested (44–47). The model shares certain key similarities with human HF—notably, neurohormonal activation (12,15), beta-adrenergic receptor downregulation (16), decreased levels of cyclic adenosine monophosphate (47,48) and altered force–frequency relations (42,49). In addition, Li et al. (32) found that, similar to human HF, there was no difference between control dogs and dogs with HF in the inducibility of VT by programmed electrical stimulation (32). The present study adds to this list a relatively high incidence of sudden death and malignant arrhythmia.

Heart failure from rapid pacing differs from human HF in several important ways. Most notable are its reversal after pacing termination and the fact that myocyte hypertrophy does not occur as HF evolves (50). The canine model offers advantages over small animal models for studying cardiac electrophysiology. For example, the hearts of small animals do not generally display sustained ventricular tachyarrhythmias sufficient for SCD, whereas the canine heart is large enough to be susceptible to death by ventricular tachyarrhythmias. Thus, although it is not a perfect substitute for human HF, the canine pacing model does share a remarkable number of similarities, making it useful for evaluating mechanisms and potential treatments to prevent SCD due to HF.

Study limitations. The measurement of $MAPD_{90}$ can be sensitive to the precise orientation of the MAP catheter and the pressure applied to the endocardium. However, we recorded MAP at multiple sites in both groups and only used tracings with a stable baseline. Furthermore, multiple beats at each site were signal averaged to arrive at the $MAPD_{90}$. This reduced the likelihood that MAP position artifact influenced our results. Lastly, the MAP data were also supported by direct APD measurement in isolated myocytes.

High doses of CsCl can markedly elevate systemic pressures by causing catecholamine release, and the resulting enhanced afterload may, in itself, cause repolarization abnormalities (22). Although we did not measure arterial blood pressures during the CsCl infusion, the doses we used would likely not have resulted in significant systemic pressure elevations. Hanich et al. (22) reported minimal increases in blood pressure after a cumulative dose of 0.825 mmol/kg of CsCl. In the present study, we found significant increases in $MAPD_{90}$ in failing hearts at a cumulative dose of only 0.75 mmol/kg of CsCl. If there were disparate effects on blood pressure rise in control dogs and those with HF, the latter would likely have had a reduced pressor response due to adrenergic receptor downregulation. The hypothesis that CsCl itself, not its hemodynamic effects, causes heightened repolarization abnormalities in HF in vivo was further supported by similar responses measured in isolated myocytes.

The scope of this study admittedly prevented us from drawing direct conclusions about the exact cause of sudden death. It was impossible to test a mechanistic link between the severity of cellular electrophysiologic changes and sudden death, because cells could only be obtained from freshly arrested hearts. Furthermore, associating the degree of chamber repolarization changes, particularly dispersion of repolarization, with arrhythmogenic death would require a substantially larger sample size. Therefore, although we did find that HF induced by tachycardia pacing in these dogs was associated

with a high incidence of sudden death, an increased incidence of ventricular arrhythmias, prolonged and heterogeneous repolarization and increased sensitivity of $MAPD_{90}$ to the repolarizing K^+ current inhibitor CsCl, we cannot establish a definitive cause and effect link between the repolarization abnormalities and sudden death from this study.

Conclusions. Tachycardia-induced HF in dogs displays marked repolarization abnormalities, including prolongation of the action potential, increased spatial dispersion of $MAPD_{90}$ and enhanced sensitivity of APD_{90} to repolarizing K^+ current blockade by CsCl. These changes are accompanied by high grade ventricular ectopy and sudden death in these animals. Together, these data form an important link between previously reported cellular repolarization defects and profound electrophysiologic abnormalities at the chamber level. As these changes bear many similarities to HF in humans, useful mechanistic insights and meaningful tests of novel treatment strategies for this vexing complication of cardiomyopathy should be feasible with this animal model.

We thank Medtronic, Inc. for graciously supplying the pulse generators and pacing leads for this study, and Del Mar Avionics for providing the Holter monitor recorders.

References

1. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and heart failure. N Engl J Med 1991;325:293–302.
2. Cohn JN, Johnson G, Ziesche S, et al. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. N Engl J Med 1991;325:303–10.
3. The SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. N Engl J Med 1992;327:685–91.
4. Stevenson WG, Stevenson LW, Middlekauff HR, Saxon LA. Sudden death prevention in patients with advanced ventricular dysfunction. Circulation 1993;88:2953–61.
5. Tomaselli GF, Beuckelmann DJ, Calkins HG, et al. Sudden cardiac death in heart failure: the role of abnormal repolarization. Circulation 1994;90: 2534–9.
6. Aronson RS. Characteristics of action potentials of hypertrophied myocardium from rats with renal hypertension. Circ Res 1980;47:443–54.
7. Gwathmey JK, Copelas L, MacKinnon R, et al. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ Res 1987;61:70–6.
8. Scamps F, Mayoux E, Charlemagne D, Vassort G. Calcium current in single cells isolated from normal and hypertrophied rat heart: effects of β-adrenergic stimulation. Circ Res 1990;67:199–208.
9. Beuckelmann DJ, Näbauer M, Erdmann E. Alterations of K^+-currents in isolated human ventricular myocytes from patients with terminal heart failure. Cir Res 1993;73:379–85.
10. Di Diego JM, Antzelevitch C. High $[Ca^{2+}]_o$-induced electrical heterogeneity and extra-systolic activity in isolated canine ventricular epicardium. Circulation 1994;89:1839–50.
11. Aronson RS, Ming Z. Cellular mechanisms of arrhythmias in hypertrophied and failing myocardium. Circulation 1993;87 Suppl VII-VII:76–83.
12. Armstrong PW, Stopps TP, Ford SE, De Bold AJ. Rapid ventricular pacing in the dog: pathophysiologic studies of heart failure. Circulation 1986;74: 1075–84.
13. Damiano RJ, Tripp HF, Asano T, Small KW, Jones RH, Lowe JE. Left ventricular dysfunction and dilatation resulting from chronic supraventricular tachycardia. J Thorac Cardiovasc Surg 1987;94:135–43.
14. Wilson JR, Douglas P, Hickey WF, et al. Experimental congestive heart failure produced by rapid ventricular pacing in the dog: cardiac effects. Circulation 1987;75:857–67.
15. Riegger AJG, Liebau G. The renin-angiotensin-aldosterone system, antidiuretic hormone and sympathetic nerve activity in an experimental model of congestive heart failure in the dog. Clin Sci 1982;62:465–9.
16. Vatner DE, Vatner SF, Fujii AM, Homcy CJ. Loss of high affinity cardiac beta adrenergic receptors in dogs with heart failure. J Clin Invest 1985;76: 2259–64.
17. Williams RE, Kass DA, Kawagoe Y, et al. Endomyocardial gene expression during development of pacing tachycardia-induced heart failure in the dog. Circ Res 1994;75:615–23.
18. Kääb S, Nuss HB, Chiamvimonvat N, et al. Ionic mechanism of action potential prolongation in ventricular myocytes from dogs with pacing-induced heart failure. Circ Res 1996;78:262–73.
19. Isenberg G. Cardiac Purkinje fibers: cesium as a tool to block inward rectifying potassium currents. Pflugers Arch 1976;365:99–106.
20. Meier CF, Katzung BG. Cesium blockade of delayed outward currents and electrically-induced pacemaker activity in mammalian ventricular myocardium. J Gen Physiol 1981;77:531–47.
21. Marban E, Tsien RW. Effects of nystatin-mediated intracellular ion substitution on membrane currents in calf Purkinje fibers. J Physiol 1982;329:569–87.
22. Hanich RF, Levine JH, Spear JF, Moore EN. Autonomic modulation of ventricular arrhythmia in CsCl-induced long QT syndrome. Circulation 1988;77:1149–61.
23. Beuckelmann DJ, Näbauer M, Erdmann E. Intracellular calcium handling in ventricular myocytes from patients with terminal heart failure. Circulation 1992;85:1046–55.
24. Antzelevitch C, Sicouri S, Litovsky SH, et al. Heterogeneity within the ventricular wall: electrophysiology and electropharmacology of epicardial, endocardial, and M cells. Circ Res 1991;69:1227–49.
25. Wettwer E, Amos GJ, Posival H, Ravens U. Transient outward current in human ventricular myocytes of subepicardial and subendocardial origin. Circ Res 1994;75:473–82.
26. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved path-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 1981;391:85–100.
27. Simpson GG, Roe A, Lewontin RC. Quantitative Zoology. New York (NY): Harcourt, Brace, Jovanovich, 1960:440.
28. Priori SG, Napolitano C, Diehl L, Schwartz PJ. Dispersion of the QT interval: a marker of therapeutic efficacy in the idiopathic long QT syndrome. Circulation 1994;89:1681–9.
29. Brachman J, Scherlag BJ, Rosenshtraukh LV, Lazzara R. Bradycardia-dependent triggered activity: relevance to drug-induced multiform ventricular tachycardia. Circulation 1983;68:846–56.
30. Levine JH, Spear JF, Guarnieri T, et al. Cesium chloride–induced long QT syndrome: demonstration of afterdepolarizations and triggered activity in vivo. Circulation 1985;72:1092–1103.
31. Wang Z, Taylor LK, Denney WD, Hansen DE. Initiation of ventricular extrasystoles by myocardial stretch in chronically dilated and failing canine left ventricle. Circulation 1994;90:2022–31.
32. Li HG, Jones DL, Yee R, Klein GJ. Electrophysiologic substrate associated with pacing-induced heart failure in dogs: potential value of programmed stimulation in predicting sudden death. J Am Coll Cardiol 1992;19:444–9.
33. Li HG, Jones DL, Yee R, Klein GJ. Arrhythmogenic effects of catecholamines are decreased in heart failure induced by rapid pacing in dogs. Am J Physiol 1993;265:H1654–62.
34. Day CP, McComb JM, Campbell RWF. QT dispersion: an indication of arrhythmia risk in patients with long QT intervals. Br Heart J 1990;63:342–4.
35. Hii JTY, Wyse DG, Gillis AM, Duff HJ, Solylo MA, Mitchell LB. Precordial QT interval dispersion as a marker of torsades de pointes: disparate effects of class Ia antiarrhythmic drugs and amiodarone. Circulation 1992;86:1376–82.
36. Perkiomaki JS, Koistinen MJ, Yli-Mayry S, Huikuri HV. Dispersion of QT interval in patients with and without susceptibility to ventricular tachyarrhythmias after previous myocardial infarction. J Am Coll Cardiol 1995;26: 174–9.
37. Glancy JM, Garratt CJ, Woods KL, de Bono DP. QT dispersion and mortality after myocardial infarction. Lancet 1995;345:945–8.
38. Buja G, Miorelli M, Turrini P, Melacini P, Nava A. Comparison of QT

dispersion in hypertrophic cardiomyopathy between patients with and without ventricular arrhythmias and sudden death. Am J Cardiol 1993;72:973–6.
39. Miorelli M, Buja G, Melacini P, Fasoli G, Nava A. QT-interval variability in hypertrophic cardiomyopathy patients with cardiac arrest. Int J Cardiol 1994;45:121–7.
40. Barr CS, Naas A, Freeman M, Lang CC, Struthers AD. QT dispersion and sudden unexpected death in chronic heart failure. Lancet 1994;343:327–9.
41. Panciera DL, Refsal KR. Thyroid function in dogs with spontaneous and induced congestive heart failure. Can J Vet Res 1994;58:157–62.
42. Wolff MR, de Tombe PP, Harasawa Y, et al. Alterations in left ventricular mechanics, energetics, and contractile reserve in experimental heart failure. Circ Res 1992;70:516–29.
43. O'Brien PJ, Duke AL, Shen H, Shohet RV. Myocardial mRNA content and stability, and enzyme activities of Ca-cycling and aerobic metabolism in canine dilated cardiomyopathies. Mol Cell Biochem 1995;142:139–50.
44. Cheng CP, Pettersson K, Little WC. Effects of felodipine on LV systolic and diastolic performance in congestive heart failure. J Pharmacol Exp Ther 1994;271:1409–17.
45. Margulies KB, Barclay PL, Burnett JC Jr. The role of neutral endopeptidase in dogs with evolving congestive heart failure. Circulation 1995;91:2036–42.
46. Evans MA, Burnett JC Jr, Redfield MM. Effect of low dose aspirin on cardiorenal function and acute hemodynamic response to enalaprilat in a canine model of severe heart failure. J Am Coll Cardiol 1995;25:1445–50.
47. Spinale FG, Holzgrefe HH, Mukherjee R, et al. Angiotensin-converting enzyme inhibition and the progression of congestive cardiomyopathy. Circulation 1995;92:562–78.
48. Roth DA, Urasawa K, Helmer GA, Hammond HK. Downregulation of cardiac guanosine 5′-triphosphate-binding proteins in right atrium and left ventricle in pacing-induced congestive heart failure. J Clin Invest 1993;91:939–49.
49. Sasayama S, Asanoi H, Ishizaka S. Continuous measurement of the pressure–volume relationship in experimental heart failure produced by rapid ventricular pacing in conscious dogs. Eur Heart J 1992;13 Suppl E:47–51.
50. Howard RJ, Stopps TP, Moe GW, Gotlieb A, Armstrong PW. Recovery from heart failure: structural and functional analysis in a canine model. Can J Physiol Pharmacol 1988;66:1505–12.